

In the Specification

Kindly delete the paragraph appearing on page 74 at line 16 through line 23 on page 75 and replace it with the following:

Cloning of a Brassica napus Seed
cDNA Encoding Seed Microsomal Delta-12
Fatty Acid Desaturase

For the purpose of cloning the Brassica napus seed cDNA encoding a delta-12 fatty acid desaturase, the cDNA insert from pSF2b was isolated by digestion of pSF2b with EcoR I followed by purification of the 1.2 kb insert by gel electrophoresis. The 1.2 kb fragment was radiolabeled and used as a hybridization probe to screen a lambda phage cDNA library made with poly A⁺ mRNA from developing Brassica napus seeds 20-21 days after pollination. Approximately 600,000 plaques were screened under low stringency hybridization conditions (50 mM Tris pH 7.6, 6X SSC, 5X Denhardt's, 0.5% SDS, 100 [μg] μg/ml denatured calf thymus DNA and 50°C) and washes (two washes with 2X SSC, 0.5% SDS at room temperature for 15 min each, then twice with 0.2X SSC, 0.5% SDS at room temperature for 15 min each, and then twice with 0.2X SSC, 0.5% SDS at 50°C for 15 min each). Ten strongly-hybridizing phage were plaque-purified and the size of the cDNA inserts was determined by PCR amplification of the insert using phage as template and T3/T7 oligomers for primers. Two of these phages, 165D and 165F, had PCR amplified inserts of 1.6 kb and 1.2 kb respectively and these phages were also used to excise the phagemids as described above. The phagemid derived from phage 165D, designated pCF2-165D, contained a 1.5 kb insert which was sequenced completely on one strand. SEQ ID NO:3 shows the 5' to 3' nucleotide sequence of 1394 base pairs of the Brassica napus cDNA which encodes delta-12 desaturase in plasmid pCF2-165d. Nucleotides 99 to 101 and nucleotides 1248 to 1250 are, respectively, the putative initiation codon and the termination codon of the open reading frame (nucleotides 99 to 1250). Nucleotides 1 to 98 and 1251 to 1394 are, respectively, the 5' and 3' untranslated nucleotides. The 383 amino acid protein sequence deduced from the open reading frame in SEQ ID NO:3 is shown in SEQ ID NO:4. While the other strand can easily be sequenced for confirmation, comparisons of SEQ ID NOS:1 and 3 and of SEQ ID NOS:2 and 4, even admitting of possible sequencing errors, showed an overall homology of approximately 84% at both the nucleotide and amino acid levels, which confirmed that pCF2-165D is a Brassica napus seed cDNA that encoded delta-12 desaturase. Plasmid pCF2-165D has been deposited on October 16, 1992 with the American Type Culture Collection of Rockville, Maryland under the provisions of the Budapest Treaty and bears accession number ATCC 69094.

Kindly delete the paragraph appearing on page 74 starting at line 13 through line 23 on page 75 and replace it with the following:

Cloning of a Brassica napus Seed
cDNA Encoding Microsomal Delta-12 Fatty Acid
Desaturase

The radiolabelled probe was used to screen a Brassica napus seed cDNA library. In order to construct the library, Brassica napus seeds were harvested 20-21 days after pollination, placed in liquid nitrogen, and polysomal RNA was isolated following the procedure of Kamalay et al., (Cell (1980) 19:935-946). The polyadenylated mRNA fraction was obtained by affinity chromatography on oligo-dT cellulose (Aviv et al., Proc. Natl. Acad. Sci. USA (1972) 69:1408-1411). Four micrograms of this mRNA were used to construct a seed cDNA library in lambda phage (Uni-ZAP™ XR vector) using the protocol described in the ZAP-cDNA™ Synthesis Kit (1991 Stratagene Catalog, Item #200400). Approximately 600,000 clones were screened for positively hybridizing plaques using the radiolabelled EcoR I fragment from pSF2b as a probe essentially as described in Sambrook et al., (Molecular Cloning: A Laboratory Manual, 2nd ed. (1989) Cold Spring Harbor Laboratory Press) except that low stringency hybridization conditions (50 mM Tris, pH 7.6, 6X SSC, 5X Denhardt's, 0.5% SDS, 100 [µg] µg/ml denatured calf thymus DNA and 50°C) were used and post-hybridization washes were performed twice with 2X SSC, 0.5% SDS at room temperature for 15 min, then twice with 0.2X SSC, 0.5% SDS at room temperature for 15 min, and then twice with 0.2X SSC, 0.5% SDS at 50°C for 15 min. Ten positive plaques showing strong hybridization were picked, plated out, and the screening procedure was repeated. From the secondary screen nine pure phage plaques were isolated. Plasmid clones containing the cDNA inserts were obtained through the use of a helper phage according to the in vivo excision protocol provided by Stratagene. Double-stranded DNA was prepared using the alkaline lysis method as previously described, and the resulting plasmids were size-analyzed by electrophoresis in agarose gels. The largest one of the nine clones, designated pCF2-165D, contained an approximately 1.5 kb insert which was sequenced as described above. The sequence of 1394 bases of the cDNA insert of pC1 2-165D is shown in SEQ ID NO:3. Contained in the insert but not shown in SEQ ID NO:3 are approximately 40 bases of the extreme 5' end of the 5' non-translated region and a poly A tail of about 38 bases at the extreme 3' end of the insert.